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STUDIES ON THE COCCACEAE

XVII. AGGLUTINATION AS A MEANS OF DIFFERENTIATING THE SPECIES OF *STREPTOCOCCUS* AND *LEUCONOSTOC*

G. J. HUCKER

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TECHNICAL BULLETIN No. 190

STUDIES ON THE COCCACEAE

XVII. AGGLUTINATION AS A MEANS OF DIFFERENTIATING THE SPECIES OF *STREPTOCOCCUS* AND *LEUCONOSTOC*[1]

G. J. HUCKER

ABSTRACT

A study has been made from the standpoint of agglutination and agglutination absorption of the relationships of certain strains of the genera *Streptococcus* and *Leuconostoc* with particular emphasis upon the relationships of the saprophytic types. The 36 strains used for the study were secured from a variety of sources and were selected with the thought of studying a representative series of authentic strains of these two genera rather than a large number of freshly isolated types. No effort was made to form serological groupings.

It was found that agglutination and agglutination absorption were not useful in all cases in differentiating species of the streptococci due in a number of instances to a decided strain specificity.

Of the saprophytic types, *Streptococcus lactis* (Lister) Löhnis, *S. cremoris* Orla-Jensen, and *S. thermophilus* Orla-Jensen appeared to be distinct from an agglutination standpoint. *S. glycerinaceus* Orla-Jensen was found to be closely related to and possibly identical with the gelatin liquefying streptococci. *S. faecium* Orla-Jensen serum was found to agglutinate strains of *S. lactis*.

The saprophytic streptococci did not give cross reactions with the more parasitic members of the genus. *Leuconostoc dextranicus* (Beijerinck) Hucker and Pederson and *L. mesenteroides* (Cienkowski) Van Tieghem showed cross reactions to a high degree with sera prepared from many strains of parasitic as well as saprophytic streptococci.

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[1]The major portion of the data reported here was secured in the laboratory of Dr. R. St. John-Brooks, Lister Institute, London, England.

INTRODUCTION

Regardless of the type of test and method of procedure, one of the underlying objects of cultural studies on individual groups should be to secure information bearing on the identity, as well as the systematic relationships, of the species included in the group. The streptococci have received considerable attention due to their importance in medicine, agriculture, and industry. Probably no other group is more closely identified with as many fields of bacteriology as the streptococci, a fact which necessitates careful consideration of their systematic relationships and a studied selection of characters which may be satisfactorily used to separate the various species.

Many routine laboratory tests have been used in separating the streptococci, some with apparent success, while others have fallen short of the hopes of those by whom they were originally outlined. The use of serological methods for the separation of the streptococci into species has received considerable attention as such reactions have proved valuable in many cases in differentiating closely related organisms, particularly those of a parasitic nature.

If serological reactions are to be used in the differentiation of species of the streptococci, then such methods should be applicable to the entire group and not limited to special groups within the genus. The object of this investigation has been to make a survey, from the standpoint of the agglutination and agglutination absorption tests, of representative strains of the genera *Streptococcus* and *Leuconostoc* in the hope that these reactions may be of value in the differentiation of the saprophytic types as well as strains that are more typically parasitic in nature.

HISTORY

A voluminous literature discusses the agglutination and agglutination absorption tests as applied to the streptococci. This literature deals primarily with the serological relationships of the parasitic rather than those of the saprophytic streptococci. Lingelsheim (1928)[2], Birkhaug (1928), and others have given full accounts of the earlier work and no effort will be made to review these investigations here.

[2]Refers to Literature Cited, page 27.

Little study has been made of the serological relationships of the saprophytic streptococci and no data have been published which discuss these relationships as applied to the species in the genus *Leuconostoc*.

METHODS

Following a survey of the various methods which are now prevalent for studying agglutination reactions, the "drop method" was adopted. This particular method is based upon the principle of preparing the serum dilution by standardized drops. These drops are maintained at an approximate constant size by using a quill tube which has been drawn out to a capillary point, the size of the outlet being determined by an ordinary wire gauge. By careful manipulation and by maintaining the pipette in a vertical position and delivering the drop slowly, reasonably accurate dilutions can be made. The limitations of this method are realized, but its advantages for the present investigation warranted its use.

Two cubic centimeters of suspension of the antigen were used to which was added the proper number of drops from the previously prepared pipette to insure the desired dilution. The antigen-serum mixtures were shaken and allowed to incubate at 37°C for 4 hours, shaken again, and then held in the ice chest over night.

Immune sera were prepared in rabbits which showed no normal agglutinins for streptococci. Certain rabbits did not react to the inoculation of the suspended organisms. In a series of 28 inoculated animals, 4 failed to produce any agglutinins from two separate strains of streptococci. These two cultures upon being inoculated into a second series of rabbits stimulated the production of sera of a relatively high titre.

The animals were inoculated on the first, fifth, seventh, eleventh, and thirteenth day. Eight days following the final inoculation, a test bleeding was made and the serum titre determined. Generally, these five inoculations yielded serum with a titre of 1 to 1,200. In one instance a titre of 1 to 2,560 had been built up during the inoculation period. A second, similar series of inoculations were used if trial bleedings showed unsatisfactory sera. The serum was preserved with tricresol.

TABLE 1.—RECIPROCAL AGGLUTINATIONS OF A REPRESENTA-

ANTIGEN	SERA PREPARED FROM*																	
	S. lactis (2)	*S. lactis* (3)	*S. cremoris* (9)	*S. cremoris* (11)	*S. thermophilus* (7)	*S. inulinaceus* (10)	*S. glycerinaceus* (8)	*L. dextranicus* (5)	*L. mesenteroides* (13)	*L. mesenteroides* (64)	*L. dextranicus* (87)	*L. dextranicus* (88)	*L. dextranicus* (84)	*S. apis* (28)	*S. liquefaciens* (15)	*S. faecium* (12)	*Enterococcus* (91)	*S. viridans* (33)
	†																	
S. lactis (2)	**2560**	80	—	—	—	1280	80	1280	—	—	160	—	160	—	1280	640	640	160
S. lactis (3)	2560	**2560**	20	—	80	80	2560	320	160	—	40	320	80	160	—	1280	640	640
S. cremoris (9)	320	—	**2560**	2560	160	640	80	320	320	280	—	—	160	640	640	—	80	—
S. cremoris (11)	—	20	640	**2560**	—	320	80	640	80	—	160	160	640	—	80	—	—	—
S. thermophilus (7)	—	—	—	—	**2560**	—	—	—	—	—	—	—	—	320	—	320	640	—
S. inulinaceus (10)	—	—	—	40	—	**2560**	1280	160	80	—	80	—	40	80	1280	640	—	—
S. glycerinaceus (8)	640	—	—	—	—	640	**2560**	—	—	—	160	—	160	640	2560	—	320	—
L. dextranicus (5)	640	320	—	—	—	320	320	**1280**	—	—	—	40	320	320	—	—	320	1280
L. mesenteroides (13)	640	320	—	—	—	640	640	320	**1280**	—	320	—	320	—	160	40	80	1280
L. mesenteroides (64)	—	—	—	—	—	—	640	—	—	**1280**	—	—	—	160	—	640	—	80
L. dextranicus (87)	320	320	40	40	80	160	1280	640	—	320	**1280**	40	40	160	640	—	—	—
L. citrovorus (88)	—	—	—	—	—	160	40	80	—	80	1280	**2560**	640	—	—	160	320	—
L. dextranicus (84)	640	320	—	—	160	160	160	320	80	1280	640	160	**1280**	160	320	—	—	—
S. apis (28)	—	—	—	—	—	—	—	—	—	—	—	—	80	**1280**	2560	—	—	—
S. liquefaciens (15)	—	—	—	—	—	640	640	—	—	—	—	—	40	1280	**2560**	640	40	—
S. faecium (12)	320	640	40	—	160	—	640	640	320	80	40	160	—	160	—	**2560**	40	1280
Enterococcus (91)	—	—	160	—	1280	—	60	—	—	—	—	—	—	80	160	160	**1280**	—
S. viridans (33)	—	—	—	—	—	—	80	—	—	—	—	—	80	—	—	—	—	**2560**
S. mitior (41)	—	—	—	40	—	—	—	40	—	—	—	160	—	—	—	—	—	160
S. equinus (40)	—	—	—	—	—	—	—	80	—	—	160	—	—	—	—	—	—	—
S. mastitidus (67)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	640	—	—
S. mastitidus (70)	—	—	40	—	160	—	—	320	—	—	—	—	—	40	80	—	—	—
S. scarlatinae (25)	—	—	—	40	—	—	—	40	—	80	—	—	—	—	—	—	—	—
S. scarlatinae (82)	—	20	—	—	—	—	—	—	—	—	—	—	—	80	—	—	80	—
S. scarlatinae (83)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	320
S. puerperalis (80)	—	—	—	—	—	—	40	—	—	—	—	—	160	—	—	80	—	80
S. puerperalis (81)	—	—	—	—	—	—	—	—	—	—	—	40	—	—	—	—	—	40
S. erysipelatos (78)	—	—	—	—	80	—	—	—	—	—	320	—	—	320	—	40	—	320
S. erysipelatos (79)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	—	—	80
S. asalignus (66)	—	—	—	—	40	—	—	320	—	—	320	80	640	—	—	—	66	—
S. epidemicus (68)	—	—	—	—	—	—	—	—	80	—	80	80	—	—	—	—	320	—
S. infrequens (65)	—	—	—	—	—	320	—	—	—	—	—	—	—	40	—	—	—	160
S. cunniculi (42)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	320	160
S. stenos (39)	20	—	—	—	—	—	80	—	—	—	—	—	—	—	—	640	—	320
S. equi (X19)	—	—	—	—	—	—	—	160	—	—	—	—	80	—	—	—	1280	40
S. anhemolyticus (128)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	160	160

*Sera produced by intravenous inoculation of suspensions of antigen into rabbits.
†Numbers denote highest dilution in which agglutination could be detected.

VE GROUP OF SPECIES OF *Streptococcus* AND *Leuconostoc*.

SERA PREPARED FROM*																		ORIGIN OF CULTURE
S. mitior (11)	*S. equinus* (40)	*S. mastitidus* (67)	*S. mastitidus* (70)	*S. scarlatinae* (25)	*S. scarlatinae* (82)	*S. scarlatinae* (83)	*S. puerperalis* (80)	*S. puerperalis* (81)	*S. erysipelatos* (78)	*S. erysipelatos* (79)	*S. asalignus* (66)	*S. epidemicus* (68)	*S. infrequens* (65)	*S. cuniculi* (42)	*S. stenos* (39)	*S. equi* (X19)	*S. anhemolyticus* (128)	
—	—	—	—	—	—	160	—	320	—	—	—	—	640	—	—	160	320	S. Orla-Jensen No. 224
20	640	—	80	—	40	320	40	320	160	160	—	—	320	80	—	320	160	S. Orla-Jensen No. 9
40	80	—	—	—	40	—	—	—	—	—	40	—	640	—	—	—	80	S. Orla-Jensen No. 24
—	—	—	—	80	—	—	80	40	—	—	—	—	1280	—	160	—	—	S. Orla-Jensen No. 23
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S. Orla-Jensen No. 2
—	—	—	—	—	—	640	80	—	—	—	—	—	40	—	—	—	320	S. Orla-Jensen No. 4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S. Orla-Jensen No. 1
60	320	—	—	160	320	160	—	160	—	320	320	—	320	320	320	—	—	S. Orla-Jensen *Betacoccus bovis* No. 33
80	320	320	160	40	40	320	40	160	160	—	—	—	640	160	—	40	40	S. Orla-Jensen *Betacoccus arabinosaceus* No. 13
60	—	—	—	160	40	—	160	160	—	160	—	—	—	—	—	40	80	S. Orla-Jensen *Betacoccus arabinosaceus* No. 12
—	—	320	—	160	—	—	80	—	—	—	—	—	—	—	—	—	—	Amer. Type Cul. Coll., *S. kefir* No. 341
20	—	—	—	—	—	40	—	—	—	—	—	—	—	—	—	—	—	Amer. Type Cul. Coll., *S. citrovorus* No. 797
—	—	80	—	80	40	—	—	—	40	80	320	—	—	—	—	—	—	Amer. Typ Cul. Coll., *S. paracitrovorus* No. 798
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Kral Collection
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	S. Orla-Jensen No. 1
60	320	640	640	320	320	320	320	320	80	320	160	—	320	320	40	640	640	S. Orla-Jensen No. 6
—	—	320	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Pasteur Institute, Paris
—	—	—	—	80	80	—	—	320	—	640	—	—	—	—	—	—	—	Kral Collection
60	80	—	—	80	80	—	80	—	80	40	—	—	80	1280	—	—	—	Mulford Antitoxin Lab.
—	2560	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Mulford Antitoxin Lab.
—	—	1280	—	80	40	—	80	—	—	—	—	—	—	—	—	640	1280	Nat. Coll. Type Cul., No. 2295
20	—	—	1280	—	—	—	—	—	—	—	80	—	—	—	—	—	—	S. Orla-Jensen No. 8
—	—	80	160	1280	640	160	—	—	—	—	—	—	—	—	—	—	—	Nat. Coll. Type Cul., No. 1892
—	—	—	—	640	2560	80	—	—	—		—	—	—	—	—	—	—	Nat. Coll. Type Cul., No. 2267
—	—	—	—	80	640	1280	—	—	320		—	320	—	—	—	40	—	Nat. Coll. Type Cul., No. 2268
—	—	—	—	160	80	—	1280	640	80	80	—	—	40	—	—	80	80	Nat. Coll. Type Cul., No. 2368
—	—	—	—	40	40	—	640	1280	—	80	—	—	—	—	—	—	—	Nat. Coll. Type Cul., No. 2369
40	—	—	—	160	40	—	40	—	640	320	—	—	—	—	—	80	—	Nat. Coll. Type Cul., No. 2365
—	—	—	—	—	320	—	—	—	160	1280	—	—	—	—	—	—	—	Nat. Coll. Type Cul., No. 2366
—	—	320	—	—	—	—	—	80	—	—	1280	—	80	—	—	—	—	Nat. Coll. Type Cul., No. 2294
—	—	—	—	—	160	—	—	—	—	80	—	640	—	—	—	—	—	Nat. Coll. Type Cul., No. 2297
—	—	—	—	40	40	—	—	—	—	80	—	—	2560	80	—	40	—	Nat. Coll. Type Cul., No. 2296
—	—	—	—	—	—	—	—	—	—	160	—	—	—	2560	—	80	—	Mulford Antitoxin Lab.
—	—	—	—	160	40	—	40	40	—	80	—	—	—	—	1280	—	—	Mulford Antitoxin Lab.
—	—	—	—	—	—	—	320	—	—	80	—	—	—	—	—	1280	—	Amer. Type Cul. Coll., No. 837
—	—	—	—	—	—	—	—	—	40	80	—	—	—	—	—	—	1280	Amer. Type Cul. Coll., No. 161

SOURCE OF STRAINS STUDIED

The 36 strains used in these studies were secured from various sources (Table 1) and were selected with the thought of securing a representative series of authentic strains of *Streptococcus* and *Leuconostoc*. No effort was made to study a large number of freshly isolated strains nor to form serological groupings. The scope of this investigation has been limited to the study of a small number of named, authentic strains characteristic of certain species of these genera. The names of the strains of *Streptococcus* used in this discussion are those attached to the cultures when they were secured and no effort has been made to redetermine their identity.

The strains of *Leuconostoc* studied have been given the names suggested by Hucker and Pederson (1930). The original name attached (Table 1) to the culture when received is also noted.

ROUGH STRAINS

Considerable difficulty was experienced during this investigation with so-called "rough strains." The problem of completing any satisfactory agglutination studies with the streptococci always has centered largely around these types which spontaneously agglutinate. It was found that many of the rough strains, when grown in a broth medium highly buffered with phosphates and transferred rapidly thru several generations, tended to remain in suspension. However, a few strains, particularly those which fermented glycerol, were never brought to the point where they would remain in suspension for any length of time. One particular strain, *viz.*, *Streptococcus glycerinaceus*, was exceedingly difficult to keep in suspension, and many methods were tried with little or no success. The only procedure which caused this particular strain to remain in suspension for even short lengths of time was as follows: The cells were washed with ether, then with cold alcohol, dried, and suspended in 0.4 per cent saline. However, even such a procedure failed to eliminate all the difficulty as several of the controls showed a decided clumping following the first incubation period of 4 hours at 37°C. Following such treatment it is probable that the protoplasm of the cells has been so changed that results secured may not be satisfactory for use.

The results as discussed below deal with smooth strains as in a few

instances only were definitely known rough strains so treated that they would remain in suspension sufficiently long to secure conclusive data.

DATA

THE *LEUCONOSTOC* TYPE

Many workers have never had a very clear conception of the systematic relationships of the genus *Leuconostoc, viz.*, those members of the Tribe *Streptococceae* which form carbon dioxide and levo-lactic acid with occasionally some dextro-lactic acid, from the fermentation of glucose. For a time they were considered as low-acid-producing types of the more common saprophytic streptococci or as strains which had lost their vitality. For the most part they were included in the genus with true streptococci or they were closely associated with them. They have been studied by only a few workers. Orla-Jensen (1919), Hammer (1920), Hucker and Pederson (1930), and others have made more or less detailed studies of the characteristics of certain types of this group. Orla-Jensen (1919) believed them to be sufficiently different from the ordinary streptococci to warrant placing them in a separate genus, and he has applied the name *Betacoccus* to this genus. Hucker and Pederson (1930) have suggested that *Leuconostoc* is the valid name which should be used in designating this group.

Evans (1918), in working with *Streptococcus kefir* which is a member of this group, pointed out the similarities between this organism and certain of the types studied by Hammer, *viz.*, *S. citrovorus* and *S. paracitrovorus*. The latter also recognized this similarity, and suggested the possibility that these organisms were identical. On the other hand, Orla-Jensen (1919), after studying strains isolated from plant sources and from milk and milk products, described two species which have certain characters in common with the types studied by Evans and Hammer.

Hucker and Pederson (1930) made a biochemical and cultural study of a series of strains of the gas-producing types, *Leuconostoc*, including *Streptococcus citrovorus*, *S. paracitrovorus*, and *S. kefir* and *Betacoccus bovis* and *B. arabinosaceus*, together with freshly isolated strains from a variety of sources. They concluded that all of these types could be grouped into three distinct species of *Leuconostoc, viz., L. dextranicus*, which included *Streptococcus paracitrovorus*, *Streptococcus kefir*, and

Betacoccus bovis; L. citrovorus, which included *Streptococcus citrovorus;* and *L. mesenteroides*, which included *Betacoccus arabinosaceus.*

Cultures of *Leuconostoc mesenteroides*, *L. citrovorus*, and *L. dextranicus* were obtained from various sources (Table 1) and anti-sera were prepared in rabbits.

Considerable difficulty was experienced in securing a potent immunizing antigen for these strains. The members of this group generally grow very slowly and scantily even on nutrient agar slopes containing digested casein as a source of nitrogen. Inasmuch as growth in a liquid culture is reduced to a minimum, difficulty was also found at the beginning in securing a sufficient number of organisms from which to prepare an immunizing suspension.

Many times after securing a sufficient number of organisms to make an acceptable suspension, it was found that these suspensions had no antigenic value. This was especially true with the unheated suspensions. In those cases where the antigen was heated to 60°C for 1 hour, a larger number of the prepared suspensions were found to be suitable for inoculation purposes.

Upon examining the results of the reciprocal agglutinations (Table 1) it was found that sera prepared from two of the strains of *Leuconostoc dextranicus* (84, 87) contained a large number of group agglutinins, particularly for the more saprophytic strains of the streptococci. The action of these two sera indicated that the strains of *Leuconostoc* may be closely related serologically to the saprophytic streptococci commonly found in milk and milk products. This relationship was also in evidence from the standpoint of the group agglutinins produced by practically all of the saprophytic milk strains of streptococci. There appeared, however, to be a much sharper line of demarkation from a positive agglutination standpoint between the parasitic and saprophytic streptococci than between the strains of *Leuconostoc* and the milk streptococci.

Leuconostoc dextranicus (5) serum produced certain rather unusual reactions. This serum agglutinated, upon repeated trials, *Streptococcus lactis* (2) antigen to the limit of the titre of the serum. Few cultural or biochemical characters could be found in which these two organisms resembled each other, but nevertheless the relationship appears rather close from a positive agglutination standpoint. This same serum agglutinated another strain of *L. dextranicus* (87) as well as a strain of *S. cremoris* (11) to a dilution of 1 to 640 or one-half the

titre of the serum. These results were corroborated by using a second series of sera.

This evidence of group agglutinins in the sera of *Leuconostoc dextranicus* (5) was also noted to a much more limited extent (Table 1) when this serum was tested against such types as strains of *Streptococcus faecium* (12), *S. mastitidus* (70) and *S. asalignus* (66).

Leuconostoc dextranicus (5) serum also agglutinated *L. mesenteroides* (64) and another strain of *L. dextranicus* (84) in a slightly lower dilution. From the standpoint of a positive agglutination, *L. dextranicus* (5) appeared to be closely related to the dextro-lactic-acid-producing saprophytic streptococci.

With certain exceptions, serum prepared from a third strain of *Leuconostoc dextranicus* (84) gave similar reactions to the serum of *L. dextranicus* (5). This relationship is especially significant when it is realized that the strain of *L. dextranicus* (84) is known as *Streptococcus paracitrovorus* and has been found by Hucker and Pederson (1928) to be identical culturally with *L. dextranicus* (5) which is called *Betacoccus bovis* by Orla-Jensen.

No agglutination could be found, however, using *Leuconostoc dextranicus* (84) serum and *Streptococcus lactis* (2) antigen, which was in contrast to the action of the *L. dextranicus* (5) serum. Also, no agglutination was noted between *L. dextranicus* (5) antigen and *L. dextranicus* (84) serum. This fact, however, may not necessarily indicate that these two strains are not closely related.

Another noteworthy observation was the inability of *Leuconostoc dextranicus* (84) serum to agglutinate *L. dextranicus* (87). On the other hand, *L. dextranicus* (87) serum agglutinated *L. dextranicus* (84) antigen to one-half the final titre of the serum. The cultural characteristics, together with the agglutination of *L. dextranicus* (84) by *L. dextranicus* (87) serum (Tables 1, 2), would indicate that these two types are identical. Agglutination absorption tests using *L. dextranicus* (84) serum and *L. dextranicus* (87) antigen were found to be unsatisfactory.

Leuconostoc dextranicus (87) serum displayed (Table 2) one very interesting characteristic in its repeated agglutination to the complete titre of the serum of *L. dextranicus* (88) (Table 1), while another strain, *L. dextranicus* (55), was not agglutinated even in the lower dilutions. Absorption tests also failed to show any evidence of relationships between *L. dextranicus* (87) serum and *L. dextranicus*

TABLE 2.—AGGLUTINATION REACTIONS OF CERTAIN STRAINS OF *Streptococcus* AND *Leuconostoc*.

ANTIGEN	SERA PREPARED FROM						ORIGIN OF CULTURE
	L. dextranicus (5)	*L. mesenteroides* (64)	*L. mesenteroides* (13)	*L. dextranicus* (87)	*L. dextranicus* (88)	*L. dextranicus* (84)	
L. dextranicus (55)	—	320	320	—	—	640	B. W. Hammer, *S. citrovorus*
L. dextranicus (88)	80*	160	—	1280	2560	640	Amer. Type Cul. Coll., No. 797, *S. citrovorus*
L. dextranicus (56)	320	160	160	—	80	1280	B. W. Hammer, *S. paracitrovorus*
L. bovis (84)	320	1280	80	640	—	1280	Amer. Type Cul. Coll., No. 798, *S. paracitrovorus*
L. bovis (87)	640	320	—	1280	—	—	W. L. Albus, *S. kefir*
Betacoccus (63)	320	640	640	160	320	160	S. Orla-Jensen, Betacoccus, No. 11
S. asalignus (66)	320	—	—	320	80	640	W. H. Frost
Strep. from cheese (85)	—	640	640	640	640	1280	American Type Cul. Coll., No. 883
Aroma (45)	—	80	—	—	—	1280	Isolated from cheese
Aroma (50)	—	160	—	160	—	160	Isolated from cream
Serum titre	1280	1280	1280	1280	2560	1280	

*Numbers denote highest dilution in which agglutination could be detected.

(55). Culturally, these two strains of *L. dextranicus* are identical with the exception of slight variations in the amount of volatile acid produced and certain other minor differences.

It is evident, as found by Hucker (1926) in the case of the micrococci, that the species of *Leuconostoc* evidence a large amount of strain specificity which makes any general conclusions from an agglutination standpoint impossible. However, the close serological relationship between certain strains of *L. dextranicus* further corroborated the biochemical and cultural findings relative to this species.

Leuconostoc mesenteroides (13) serum gave no appreciable agglutination of *L. dextranicus* (5). This may indicate that the arabinose-fermenting strains are different, as Orla-Jensen (1919) suggests, from the *L. dextranicus* (5) types which rarely attack the pentoses. *L. dextranicus* antigen also failed to absorb agglutinins from *L. mesenteroides* serum.

The serum prepared from *Leuconostoc mesenteroides* (64), a strain which appeared in many ways to be typical of the slime-producing types from sugar, agglutinated suspensions of *L. dextranicus* (87) and *L. dextranicus* (84), altho reciprocal reactions were not found. However, a second strain (Table 2) of *L. dextranicus* (56) was agglutinated in a lower dilution by the same serum.

Such discrepancies, altho common in agglutination studies of the cocci, make impossible the drawing of any definite conclusions as to their relationships from a serological (agglutination) standpoint. Certainly there were no sharp or clear-cut division lines between the species of the genus *Leuconostoc* and the more common saprophytic dextro-lactic-acid-producing strains. Biochemically and culturally, on the other hand, certain evident characters indicate that these two groups are sufficiently distinct to warrant placing them in separate genera, as suggested by Orla-Jensen (1919).

THE NON-LIQUEFYING SAPROPHYTIC STREPTOCOCCI GENERALLY ASSOCIATED WITH MILK AND MILK PRODUCTS

Altho serological data have proved to be helpful in identifying the species of parasitic and pathogenic streptococci, little effort has been made to use such means of differentiating the more or less common types of streptococci associated with milk and milk products. The

latter streptococci have been studied culturally in detail by various investigators and from the results obtained it is now possible to secure a more or less definite picture of the different species or types of the so-called lactic acid streptococci. These species have been differentiated entirely upon cultural and biochemical characteristics. Orla-Jensen (1919) has shown that their action upon certain test sugars, as well as their ability or inability to attack casein and certain other characteristics, are sufficiently important to separate them into well-defined species. These results have been partially confirmed by Hucker (1928), and the six species or groups can be generally recognized by such cultural methods.

Strains representing four of these species were available for serological study. It was soon noted that a serum with a high titre could easily be produced from these saprophytic types and that agglutination studies can readily be carried out.

The two strains of *Streptococcus lactis* (2, 3) were characterized by a failure to ferment sucrose, raffinose, and rhamnose, while acid was produced from maltose, dextrin, and salicin. Both strains were found to be fairly active in partially breaking down casein in milk cultures.

These two strains (Table 1) showed reciprocal agglutinations in all instances, with one exception, to the complete titre of the serum. In the instance where the negative result was secured, i.e., a failure of *Streptococcus lactis* (3) serum to agglutinate *S. lactis* (2), further tests showed that *S. lactis* (2) absorbed the agglutinins from the *S. lactis* (3) serum. These two strains, altho isolated from different sources, are no doubt identical. This cross agglutination indicates that the characters as adopted for defining *S. lactis* embrace a homogenous group.

The two strains of *Streptococcus cremoris* (9, 11) showed practically complete reciprocal agglutinations which indicated that the long chain-producing types known as *S. cremoris* may comprise a more or less homogenous group.

There has been some discussion as to the relationships between *Streptococcus lactis* and *S. cremoris*. Many European investigators have insisted that the long-chained saprophytic streptococcus with the characteristic morphology of the individual unit is distinct from the paired type which normally sours milk. No cross agglutination could be secured in any instance between the four strains of S. *lactis* (2, 3) and *S. cremoris* (9, 11). These results have been partially

corroborated by agglutination absorption tests (Table 3) in which it was found that an *S. cremoris* (11) antigen failed in all instances to absorb agglutinins from *S. lactis* (3) serum. Such results would indicate that *S. lactis* and *S. cremoris* are at least two distinct varieties or types.

However, further tests (Table 4) with additional strains have shown a certain amount of cross agglutination. *Streptococcus cremoris* (9) serum agglutinated in relatively high dilutions *S. lactis* (92, 135) and a strain labelled *Bacterium güntheri* (136). These strains have been found by Hucker (1928) to ferment sucrose and maltose and thus culturally are not identical with either *S. lactis* or *S. cremoris*.

The relationship between *Streptococcus lactis* and *S. thermophilus* has also been under discussion for some time as many believe the sucrose-fermenting *S. thermophilus* types are nothing more than varieties of *S. lactis*. Such was not found to be the case if a failure to give cross agglutination may be used as an indication of their relationship. In no instance was any cross agglutination secured between strains of *S. thermophilus* and strains of *S. lactis* which were found by cultural tests to be characteristic of this species. These negative results, however, have not been verified by agglutination absorption tests.

Two other strains of these saprophytic types of streptococci, *viz.*, *Streptococcus inulinaceus* and *S. glycerinaceus*, did not give as clear-cut and conclusive results as were found with the other strains of saprophytic streptococci. These two strains showed a large amount of non-specific agglutination with other closely related strains. From the results as obtained in this study, one would hesitate to assert that these two organisms are distinct and that they represent definite and well-defined species. However, their cultural characteristics may indicate that there is a sufficient difference between the two strains and the species which they represent and other closely related strains to designate them as separate species.

A close relationship has been pointed out between *Streptococcus fecalis* and *S. lactis*. From the standpoint of agglutination absorption (Table 3), this relationship is not apparent. In no instance were *S. lactis* (3) agglutinins absorbed by either of two strains of *S. fecalis* (17, 18) or a strain of *Enterococcus* (91). Such results may indicate that *S. fecalis* and *S. lactis* are two distinct species and that the cultural characters designated to separate these two species have been well selected.

TABLE 3.—THE ABILITY OF THE VARIOUS STREPTOCOCCI

STRAIN	AGGLUTINATION IN UNABSORBED *S. lactis* (3) SERUM				ABSORBED BY
	320	640	1280	2560	
S. fecalis (17)	++	—	—	—	*S. fecalis* (17)
S. fecalis (18)	—	—	—	—	*S. fecalis* (18)
Enterococcus (91)	++	++	—	—	*Enterococcus* (91)
S. cremoris (11)	—	—	—	—	*S. cremoris* (11)
L. dextranicus (88)	—	—	—	—	*L. dextranicus* (88)
L. dextranicus (84)	++	—	—	—	*L. dextranicus* (84)
S. lactis (137)	—	—	—	—	*S. lactis* (137)
S. lactis (135)	—	—	—	—	*S. lactis* (135)
S. lactis (93)	—	—	—	—	*S lactis* (93)
B. güntheri (136)	—	—	—	—	*B. güntheri* (136)
L. dextranicus (55)	—	—	—	—	*L. dextranicus* (55)
S. lactis (92)	—	—	—	—	*S. lactis* (92)
S. lactis (3)	+++	+++	+++	+++	*S. lactis* (3)

Upon the basis of these agglutination and agglutination absorption reactions, it may be concluded that several of the species of saprophytic lactic-acid-producing streptococci are serologically distinct. On the other hand, a study of a larger number of strains may indicate that rather than these results showing specific differences, varietal or even strain characteristics are being demonstrated. This is especially true of the strains of *Streptococcus cremoris*, *S. lactis*, and *S. thermophilus* which were studied. Further cultural work is needed in order to establish the true specific relationships of these types.

THE GELATIN-LIQUEFYING STREPTOCOCCI

The gelatin-liquefying types of streptococci have received far less attention than the non-liquefying species, as these proteolytic strains are not generally encountered in routine work. Many investigators draw very indefinite lines between them and the gelatin-liquefying micrococci. The latter group, however, may be easily designated by

TO ABSORB AGGLUTININS FROM *S. lactis* (3) SERUM.

AGGLUTINATION ABSORBED SERUM								CONTROL	ORIGIN OF CULTURE
Absorbing strain				Serum strain					
320	640	1280	2560	320	640	1280	2560		
—	—	—	—	+++	+++	+++	+++	—	Nat. Coll. Type Cul., No. 371
—	—	—	—	+++	+++	+++	+++	—	Nat. Coll. Type Cul., No. 370
—	—	—	—	+++	+++	+++	+++	—	Pasteur Institute
—	—	—	—	+++	+++	+++	+++	—	S. Orla-Jensen, No. 23
—	—	—	—	+++	+++	+++	+++	—	Amer. Type Cul. Coll., No. 797
—	—	—	—	+++	+++	+++	+++	—	Amer. Type Cul. Coll., No. 798
—	—	—	—	+++	—	—	—	—	Nat. Coll. Type Cul., No. 1551
—	—	—	—	+++	+++	—	—	—	Nat. Coll. Type Cul., No. 662
—	—	—	—	+++	—	—	—	—	Kral Collection, No. 246
—	—	—	—	+++	+++	—	—	—	Nat. Coll. Type Cul., No. 661
—	—	—	—	+++	+++	+++	+++	—	B. W. Hammer, *S. citrovorus*
—	—	—	—	+++	—	—	—	—	Kral Collection, No. 98
—	—	—	—	—	—	—	—	—	S. Orla-Jensen, No. 9

their characteristic abundant surface growth in contrast to the scanty growth of the streptococci.

Of the gelatin-liquefying streptococci, *Streptococcus liquefaciens* and *S. apis* are the two most commonly discussed. The liquefying types included under *S. liquefaciens* also include culturally the gelatin-liquefying streptococcus types isolated from milk and milk products by Orla-Jensen (1919).

It is probable that all of these types of *Streptococcus liquefaciens* are identical and that they are closely related or identical culturally with *S. apis*. Agglutination studies of typical strains of *S. apis* (28) and *S. liquefaciens* (15) showed these two strains to be strikingly similar. Reciprocal agglutinations were secured (Table 1) with sera prepared from each strain to the full titre of the serum. These agglutinations corroborate the cultural results which have previously indicated that these two groups of liquefying streptococci are identical.

From the cultural standpoint, *Streptococcus glycerinaceus* is practically indistinguishable from *S. liquefaciens*, with the exception of one characteristic, *viz.*, liquefaction of gelatin. Both of these strains

TABLE 4.—AGGLUTINATION REACTIONS OF CERTAIN TYPES OF STREPTOCOCCI.

ANTIGEN	SERA PREPARED FROM					ORIGIN OF CULTURE
	S. faecium (12)	*S. cremoris* (9)	*S. thermophilus* (7)	*S. lactis* (3)	*Enterococcus* (91)	
S. fecalis (17)	—	320	—	320	1280	Nat. Coll. Type Cul., No. 371
S. fecalis (18)	160*	40	—	—	640	Nat. Coll. Type Cul., No. 370
Enterococcus (91)	160	160	1280	640	1280	Pasteur Institute
S. lactis (4)	—	640	1280	1280	1280	S. Orla-Jensen, No. 206
S. lactis (93)	640	—	—	160	—	Kral Collection, No. 246
S. lactis (92)	—	320	640	—	—	Kral Collection, No. 9897
S. lactis (86)	40	—	—	—	—	Amer. Type Cul. Coll., No. 559
S. lactis (137)	320	80	640	80	—	Nat. Coll. Type Cul., No. 1551
S. lactis (135)	—	320	320	40	1280	Nat. Coll. Type Cul., No. 662
B. güntheri (136)	640	1280	320	160	1280	Nat. Coll. Type Cul., No. 661
S. cremoris (6)	—	640	80	40	80	S. Orla-Jensen, No. 25
S. cremoris (9)	—	2560	160	—	80	S. Orla-Jensen, No. 24
S. cremoris (16)	—	80	—	80	—	S. Orla-Jensen, No. 18
S. cremoris (11)	—	640	—	—	—	S. Orla-Jensen, No. 23
L. dextranicus (88)	40	—	—	—	40	Amer. Type Cul. Coll., No. 797
Strep. from cheese (85)	40	—	40	—	40	Amer. Type Cul. Coll., No. 883
Strep. from cheese (90)	640	—	160	1280	—	Amer. Type Cul. Coll., No. 882
Serum titre	2560	2560	2560	2560	1280	

*Numbers denote highest dilution in which agglutination could be detected.

produce relatively large amounts of acid from glycerol and both have many characteristics in common to the exclusion of many of the other streptococci. The close relationship between these two strains is further emphasized by the fact that the serum of *S. liquefaciens* agglutinated a typical *S. glycerinaceus* (8) culture to the complete titre of the serum. Moreover, a serum prepared from *S. glycerinaceus* with a titre of 1 to 2,560 agglutinated *S. liquefaciens* to a dilution of 1 to 640.

The sera prepared from both *Streptococcus apis* and *S. liquefaciens* contained a large number of group agglutinins for other saprophytic streptococci as many of the common milk types were agglutinated in relatively high dilutions by these two sera.

Gorini (1910, 1926) has pointed out that the organisms which he has designated as *Caseococcus*, *Mammococcus*, *Gastrococcus*, etc., are identical with other gelatin-liquefying or proteolytic cocci, regardless of their morphological and cultural characteristics. In an earlier report by Hucker (1928), it has been shown that the three types described by Gorini are typical micrococci and should be included with the well-recognized species of the genus *Micrococcus*. Closely related to these types and practically identical with them is the species designated as *Tetracoccus casei* by Orla-Jensen (1919).

TABLE 5.—AGGLUTINATION REACTIONS OF CERTAIN GELATIN-LIQUEFYING MICROCOCCI AND STREPTOCOCCI.

ANTIGEN	SERA		ORIGIN OF CULTURE
	S. liquefaciens (15)	*S. apis*	
Tetracoccus casei (1)	——	——	S. Orla-Jensen, No. 9
Caseococcus (36)	——	——	C. Gorini, No. 41-C3
Mammococcus (37)	——	——	C. Gorini, No. 3
Gastrococcus (35)	——	——	C. Gorini, No. G2-30
S. gracilis (31)	640*	40	Kral Collection
S. apis (129)	——	160	Kral Collection, Dahlem
Emmentaler coccus (156)	——	640	Nat. Coll. Type Cul., No. 555
S. glycerinaceus (8)	2560	640	S. Orla-Jensen, No. 1
Serum titre	2560	1280	

*Numbers denote highest dilution in which agglutination could be detected.

Contrasted with these gelatin-liquefying micrococci is the *Streptococcus apis* or *S. liquefaciens* type. In the present investigation (Table 5) it was found that the sera of *S. liquefaciens* and *S. apis* failed to agglutinate the organisms which are culturally typical

micrococci in any of the dilutions. On the other hand, the sera prepared from these gelatin-liquefying streptococci generally agglutinated other liquefying streptococci. These results further indicate that generic and specific differences in the liquefying cocci cannot be based on habitat, as has been suggested by Gorini and others. Any satisfactory classification depends upon cultural characteristics and a differentiation in morphology.

THE HEMOLYTIC STREPTOCOCCI

One hesitates to enter the current discussion of the serological relationships of this group of streptococci. To those who believe that serological reactions are of paramount importance in distinguishing species, the results of the various investigations in this field are disappointing. There are many workers who feel that definite positive or negative relationships, from a serological standpoint, should be found between the various types of *Streptococcus pyogenes*, or else that the group should remain in its present chaotic condition.

Positive agglutination tests should indicate close relationships, whereas negative results have no significance. Many investigators have divided groups into species on unsubstantiated personal opinion; and once these species are set up, they spend considerable effort in endeavoring to find laboratory justification in the form of definite data to substantiate their earlier ideas. The streptococci should be arranged in a small number of species, regardless of personal inclinations, until conclusive data warrant subdivision into additional species. Splitting of earlier recognized groups into smaller subdivisions should follow rather than precede the securing of confirming data. Groups called species should not be established unless they can be definitely bounded by recognizable, significant and constant characters.

The hemolytic strains were included in this investigation in order to study their general agglutination relationships to the more saprophytic types. It should again be stated that no effort was made to study a number of these types, but rather the relationships of a few authentic strains.

Two strains of so-called *Streptococcus mastitidus* were studied. One of these strains (70) was isolated and studied culturally by Orla-Jensen (1919), while the other was secured from the National Collection

of Type Cultures in London. Both were active in splitting sodium hippurate and culturally were practically identical. The two strains showed no cross agglutination or agglutinin absorption.

The sera of both of these strains of *Streptococcus mastitidus* gave a slight agglutination with *S. faecium* (12) as well as with *Leuconostoc mesenteroides*. However, *S. faecium* (12) sera agglutinated to 1 to 640 dilution *S. mastitidus* (67) antigen, while very slight reciprocal agglutinations were noted with *S. asalignus*. A rather striking cross agglutination was found between *S. equi* and *S. anhemolyticus* sera and *S. mastitidus* (67) antigen. This relationship has not been confirmed, however, by any cultural resemblances.

The small amount of cross agglutination between *Streptococcus asalignus* and *S. mastitidus* may be indicative of certain relationships as these two strains show cultural similarities. *S. asalignus* differs, according to Frost, *et al.* (1927), culturally from *S. mastitidus* only in a failure to ferment salicin and shows similarities in producing a high final H-ion concentration in dextrose broth in addition to being able to hydrolyze sodium hippurate. This latter character has been assumed by many to designate certain of the udder hemolytic streptococci.

Another of the hemolytic streptococci which may be closely related to the two above organisms is *Streptococcus epidemicus*, an organism reported as the cause of septic sore throat when this condition occurs in epidemic form. Altho ordinarily found in the udder previous to and during a milk-borne epidemic of septic sore throat, this organism is usually thought to be introduced into the udder from a human source. Recently, considerable work has been done in an endeavor to find methods of differentiating *S. epidemicus* from the bovine, non-human pathogenic *S. mastitidus*. Many workers have assumed that the two organisms are identical, but the work of Ayers (1922) indicates that a splitting of sodium hippurate, as well as differences in the amount of acid produced from glucose, are definite characters whereby these two probably closely related hemolytic streptococci may be separated.

Three strains of *Streptococcus epidemicus* were studied. It was found that the ability of these strains to produce antibodies when inoculated into test animals was very limited. Two of the strains failed to produce appreciable amounts of agglutinins when inoculated into the three different series of rabbits. However, one of the strains (68),

after repeated inoculations into two test animals, produced a serum with a titre of 1 to 640. This serum was highly specific and failed to agglutinate, with but one exception, any other of the streptococci which were studied. On the other hand, *S. epidemicus* antigen was agglutinated by a number of other streptococcus sera. In no instance was cross agglutination noted between *S. epidemicus*, *S. asalignus*, and *S. mastitidus*. The two latter strains also failed to absorb agglutinins from the *S. epidemicus* serum.

Many workers familiar with the clinical aspects of streptococcic infections believe that the various hemolytic types produce definite clinical results. On the other hand, it is assumed by others that *Streptococcus epidemicus* is identical with *S. scarlatinae* and that the latter organism under the proper conditions may be the cause of milk-borne epidemics of septic sore throat. Present investigations indicate that there may be a very close relationship between *S. epidemicus* and certain strains of *S. scarlatinae*. Scamman (1929) describes the presence of streptococci of both scarlatiniform and epidemicus types in a septic sore throat epidemic in Plymouth, Mass., while in Charlton, Mass., evidence pointed to an epidemic traceable to scarlatiniform streptococci.

The serum prepared from *Streptococcus epidemicus* (68), altho having a titre of only 1 to 640, agglutinated *S. scarlatinae* (83) in a dilution of 1 to 320, while on the other hand, *S. scarlatinae* (82) agglutinated *S. epidemicus* in a dilution of 1 to 160. These results indicate a probability that these two organisms may prove to be identical or varieties within a species, and that the present search for tests whereby they may be separated will eventually prove futile.

At present there is considerable discussion as to the relationships of three clinically well-defined groups of hemolytic streptococci, *viz.*, *Streptococcus scarlatinae*, *S. puerperalis*, and *S. erysipelatos*. Certain English and many American investigators believed that these three types of streptococci are practically identical and have shown that the toxins produced from each of these three groups can be neutralized by antitoxins produced from all other toxin-producing streptococci. On the other hand, conclusive cross agglutinations which would support claims that these three types of streptococci are identical have not been forthcoming. However, cross agglutination has been obtained in a fairly high degree and it has been assumed that the failure to secure complete cross agglutination is due to varietal differences. Birkhaug (1928) has reported that the antitoxin pro-

duced from certain strains of *S. erysipelatos* has a decided effect in checking the special rash found in erysipelas in contrast to antitoxins produced from other groups of toxin-producing streptococci. He has found that antitoxins produced from *S. scarlatinae* and *S. puerperalis* strains fail to have any therapeutic effects in erysipelas cases. From his results he has assumed that these three groups of hemolytic streptococci must be considered as specifically distinct. He has reviewed the current discussion of this problem in detail.

Considerable difficulty was experienced in securing conclusive data on the agglutination relationships of these three groups of organisms. The chief difficulty encountered was the tendency of the *Streptococcus scarlatinae* strains in particular to agglutinate spontaneously. In this group it was noted that a rapid transfer thru several generations in a highly buffered broth aided materially in keeping the organisms in suspension. However, upon the addition of the agglutinating sera the organisms were in many instances immediately clumped into a precipitate which was not a characteristic agglutination, but had the appearance of a physical clumping. This phenomena made the results as obtained very unsatisfactory in many instances, and only those cases in which conclusive agglutination was obtained have been recorded.

A surprising amount of non-specificity was noted (Table 1) in studying the six sera prepared from these three groups of closely related hemolytic streptococci. This non-specificity was particularly noticeable when the results over the entire reciprocal agglutination studies are analyzed, and are not applicable to the results as secured between the individual strains of the group. The three strains of *Streptococcus scarlatinae*, altho showing a certain amount of cross agglutination, were not identical from an agglutination standpoint. The sera prepared from these three scarlet fever strains failed to agglutinate in high dilution the antigen of either *S. puerperalis* or *S. erysipelatos*. However, a certain amount of group agglutinins was noted. The sera prepared from *S. puerperalis* and *S. erysipelatos*, on the other hand, showed a greater amount of specificity with relation to *S. scarlatinae* as in this instance practically no group agglutinins could be demonstrated.

From these results it may be concluded that the members of these three groups of hemolytic streptococci are very closely related and that if differences are eventually established, it appears probable that they will be of a clinical nature.

THE *STREPTOCOCCUS VIRIDANS* GROUP

The *Streptococcus viridans* group of streptococci does not form a distinct sub-group of the genus *Streptococcus* as do the hemolytic species. This is especially true in light of the close relationship between certain of the fecal streptococci and the non-hemolytic normal milk-souring types. Many strains of *S. lactis* produce a typical methemoglobin zone around the colonies on blood agar plates, but they rarely hemolyze. However, the species which are generally included with the green-producing streptococci are more or less distinct and are based upon relatively well-defined cultural characteristics.

Sera produced from five typical members of this group (Table 1) showed a large amount of non-specificity. This non-specificity is particularly noticeable in the cross agglutination between various members of this *Streptococcus viridans* group and the non-hemolytic saprophytic types secured from milk and milk products.

Enterococcus (91), a culture of the original organism as described by Tissier and Martelly (1902), and *Streptococcus faecium* showed practically no cross agglutination. These two strains are considered by many to be identical. However, there are also certain cultural characteristics, as pointed out by Hucker (1928), whereby these two species can be separated. *S. faecium* failed to ferment raffinose, while *Enterococcus* (91) was found to belong to the raffinose-fermenting group. This differentiation upon sugar fermentation may prove to be of more than minor importance. It has also been noted in addition that *Enterococcus* generally produced growth on media more closely resembling a micrococcus than does *S. faecium*.

Streptococcus viridans (33), a strain secured from the Kral collection, produced a serum which was noticeably non-specific. This serum agglutinated many of the saprophytic milk streptococci, including the two type strains of *S. lactis*, as well as both *Leuconostoc dextranicus* and *L. mesenteroides*. In addition it agglutinated in the lower dilutions many of the hemolytic strains.

These agglutination results would indicate, if the strains studied are typical of this group of streptococci, that these green-producing streptococci are a transition group between the hemolytic and non-hemolytic forms. Repeated trials with the sera produced by these strains gave the most irregular results of any of the sera studied.

DISCUSSION

The use of the agglutination reaction as a means of differentiating the streptococci indicates that as a test it has certain limited value in separating species. Where positive agglutination is obtained, it is evident that the two strains of streptococci are closely related and probably identical. Reciprocal positive agglutinations further substantiate this relationship. On the other hand, negative agglutinations, even when further corroborated by agglutination absorption tests, appear to have but little value in separating species.

Many investigators have insisted and have made every effort to secure a definite classification of the streptococci by using serological methods. They have felt that, if a definite system could be based upon agglutination or similar methods, the resulting groups would be sufficiently clear cut to designate them as distinct species. From the information at hand at the present time, it appears highly unlikely that serological methods can ever be used for separating the streptococci into clearly defined species. The streptococci produce a highly specific serum, such as has also been found by Hucker (1926) in the micrococci. This indicates that serological methods have their greatest importance in separating strains rather than species. When highly specific methods are used in studying the individual strains or groups in this definite group, the results should be interpreted as indicating strains and varieties only, rather than to assume that such strains are the centers of larger groups called species. If systematic bacteriologists were to form species on the serological differences found among streptococci, the number of species would become legion and almost every freshly isolated strain would form the nucleus of a new species.

This strain specificity which is prevalent among the streptococci precludes the general use of agglutination and other serological methods in determining definite and well-defined groups which may be conveniently called species. On the other hand, many streptococci altho producing group agglutinins, do not produce agglutinins common to a group of varieties which have culturally been grouped into a species.

However, as is stated above, in dealing with the streptococci the agglutination test has certain decided advantages in aiding the study of relationships of individual strains and may serve as an aid in cataloging a new strain about which little is known in regard to its cultural characters.

CONCLUSIONS

A study has been made of a representative number of type strains of the genus *Streptococcus* and *Leuconostoc*.

Agglutination and agglutination absorption tests do not appear to be useful in recognizing species of streptococci due in many cases to a decided strain specificity. Serum groups especially among the hemolytic types did not include organisms which possess common cultural characteristics.

Strains designated as *Streptococcus lactis*, *S. cremoris*, and *S. thermophilus* appeared to be serologically distinct. *S. glycerinaceus* was found to be closely related to and possibly identical with the gelatin-liquefying streptococci. Further cultural data may, however, indicate that these differences are not of a specific nature.

Leuconostoc mesenteroides and *L. dextranicus* failed to show conclusive cross agglutination and appeared to be specifically distinct.

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